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REPRODUCTIVE CYCLES AND SUPERFETATION IN PŒCILIÏD FISHES

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Introduction

Reproductive cycles in the pœciliïd fishes are of unusual interest for three reasons: (1) this family of fresh-water fishes arose in the tropics and for the most part has remained under tropical and sub-tropical conditions where the great seasonal variations in light and temperature found in temperate and arctic zones do not exist; (2) all the species in the family are ovoviviparous. The embryos have fairly large yolk sacs and are retained in the ovarian follicles after fertilization until birth, necessitating a respiratory exchange between parent and embryos. The ovary consequently serves the double function of providing the female gametes and of providing a site and the proper conditions for retaining growing embryos; (3) superfetation occurs in some of the genera. In some instances two broods of embryos of different ages are present in the ovaries and in the most extreme case there may be at one time as many as six small separate broods.

Pœciliïd fishes have been reared in aquaria for years and some of the general facts concerning size of broods, intervals between broods, correlation between size of brood and female parent, variation of brood size and intervals between broods at the different seasons of the year, are well known. Bits of reliable information concerning reproduction in a number of genera and species are found in most of the popular books on aquarium fishes. One species in particular, *Gambusia affinis,* has been introduced into new areas in the tropics as a mosquito eradicator and its life history and reproduction have been well studied. Such studies have given relatively little attention, however, to the developing ovocytes within the ovary and the relation between these groups of ovocytes and the broods.

In order to study reproductive cycles effectively it has been found necessary to know the stages of development of the embryos and of the ovocytes within the ovary and to correlate this information with the records of the reproductive cycles. Furthermore, it is necessary to have as much of this information as possible from single females. Consequently, as far as possible, it has been the procedure to secure the brood-bearing record of an individual and then kill the individual at a definite period after the bearing of a brood in order to examine the embryos and developing ovocytes in the ovary. By following this practice throughout the year data have been secured upon the following relations: (1) the total number of broods produced within a year; (2) the relative time interval between broods; (3) the relative size of broods; (4) the exact time of fertilization of a group of eggs as related to the birth of a previous brood and as related to younger ovocytes; (5) the period of time over which fertilization takes place; (6) the variation in stage of development within a single brood; (7) whether or not fertilization occurs so as to form a new brood before the former brood is voided (superfetation); (8) the relation of the interval between broods and of the size of broods to environmental factors.

Any large collection of gravid females made in the field furnishes material for a study of the stages in the ovocytes as related to the age of the embryos being retained in the ovary. Superfetation if it exists can also be studied in this type of material.

In the present study the following species have been studied by the method of maintaining laboratory cultures and dating and dissecting the gravid females: *Gambusia affinis, Gambusia holbrookii, Gambusia panuco, Mollienisia sphenops, Mollienisia latipinna, Xiphophorus hellerii, Xiphophorus strigatus, Pseudoxiphophorus bimaculatus, Platypœcilus maculatus, Platypœcilus variatus, Lebistes reticulatus, Limia vittata, Heterandria formosa, Micropœcilia picta, Micropœcilia paræ, Pœcilistes pleurospilus* and *Quintana atrizona*. The following have been studied by the use of large numbers of living specimens in the field: *Xiphophorus hellerii, Xiphophorus strigatus* and *Pseudoxiphophorus bimaculatus,* at Cordoba, Mexico (March, 1932); *Pœciliopsis infans* at Octolan, Mexico (April, 1932); *Mollienisia sphenops* and *Gambusia panuco* near Monterey, Mexico; *Mollienisia latipinna* and *Gambusia affinis* in Louisiana. Another field study was made of *Brachyrhaphus episcopi* and *Mollienisia sphenops* at Barro Colorado Island in the Panama Canal Zone during the summer of 1936.

The writer is indebted to Dr. Stillman Wright for a large collection of *Pœcilia vivipara* from Parahyba, Brazil.

Reproductive Cycles

Various species of pœciliïds have been observed for years in aquaria and the observation has been made many times in some species that once a female attains sexual maturity she produces broods at regular intervals of about thirty days. All cyclical character seems to have disappeared in the reproduction of the male, the production of sperm being practically continuous after sexual maturity is attained. In other species it has been noted that the regular interval between broods is approximately fifteen days, while in still another species the young are born with less regularity, in smaller numbers and with less interval between the broods. Various theories have been advanced to account for the different lengths of cycles.

Some few observations have been made of brood production under natural conditions where it has been noted that the brood interval is much the same as that observed in the aquarium-reared specimens. A marked retardation of reproduction occurs during the winter months and it is much more marked under natural conditions than in aquarium-reared specimens.

It has been noted also that mature specimens obtained in the field and introduced into the laboratory do not produce broods with the regularity of laboratory-reared specimens. The broods of the wild specimens tend to be produced at longer and more irregular intervals even after the females have been adapted to laboratory conditions for periods of fifteen months. The young of these same females, reared from the first in the laboratory, produce broods with more regularity. The true and complete picture of the reproductive cycle as it occurs under natural conditions can only be obtained from material living under natural conditions and for this purpose collections of considerable size are being made at monthly intervals of *Gambusia affinis* at Key West, Florida, and of *Brachyrhaphis episcopi* in the Panama Canal Zone. A study of the ovocytes and the embryos in the gravid ovaries will make it possible to indicate the events in the reproductive cycle for all seasons.

Reproduction without Superfetation

In some species the birth of a brood is accomplished before any younger group of ova has matured sufficiently to be fertilized. Consequently only one brood will be found in the ovary at one time and the embryos will be in about the same stage of development. However, the extent to which younger cells have developed at the time the embryos are evacuated varies greatly and the following account will begin with those species in which the next younger group of cells is quite small and relatively undifferentiated.

Gambusia Type.—In *Gambusia*, females produce broods at about thirty-day intervals during late spring and summer. When eggs have been fertilized and during the time in which the embryos are developing, the cells of the next wave are small. They remain small and latent until embryos have been voided and then grow and differentiate rapidly until ready for fertilization. In an ovary containing a brood of very young embryos the oldest and largest cells are about .25 mm. in diameter. Approximately two weeks later, when the embryos are about to be born, the cells are still small, being only .3 mm. in diameter. Two or three days after the birth of the embryos these same cells have increased to a diameter of .7 to 1.5 mm., almost the maximal size attained at fertilization. Fertilization occurs over a period of about five days, beginning about seven days after the voiding of the last brood of embryos. Due to the length of the period of fertilization the embryos of a single brood may be relatively far apart in their developmental stages. In all other species examined the fertilization period is short and the embryos show less range in stages of development. *Gambusia affinis, G. holbrookii* and *G. panuco* are all very similar in the peculiarities described above.

A number of studies have been made upon the reproductive cycles of *Gambusia affinis* and *Gambusia holbrookii* (Barney and Anson, 1921*a*; Hildebrand, 1917; Kuntz, 1913; Seal, 1911; Seale, 1917). The work was concerned principally with the number of broods produced by wild specimens each year and the external factors which affect fecundity. As a result of these studies it was learned that the interval between broods in *Gambusia affinis* was about thirty days during the spring months and as long as eighty-five days during the winter if the specimens were kept in the laboratory and supplied with adequate food and warmth. It was also learned that under natural conditions several broods were produced during spring and summer but that reproduction ceased altogether during the winter. Barney and Anson correlated their study of the brood-producing record with the finding of embryos of different stages of development in the ovary and concluded: (1) that a single annual reproductive cycle occurs in *Gambusia* in its natural habitat; (2) that fertilization of all the eggs for an annual cycle occurred at the same time; (3) that the small cells in the ovary did not develop to maturity during the year, and (4) that after fertilization and partial development the embryos did not proceed at the same rate, a sufficient number to form one brood reaching the birth stage, being evacuated and being followed at approximately monthly intervals by other batches of embryos until all eggs that had been fertilized were voided.

Kuntz (1913), on the other hand, assumed that "A considerable number of ova reach maturity at the same time. These, being fertilized, give rise to a brood of young. After the birth of this brood another lot of ova reach maturity and, being fertilized, give rise to a second brood. Thus, perhaps, all the ova required for the several broods which are born during a spring and summer may be present in the ovary at the beginning of the season."

The writer is inclined to agree with Kuntz that different groups of ova reach maturity at different times and that each group is fertilized separately, for two reasons: first, all the young are born at practically the same time and none remain in the ovary; second, within ten days after birth another lot of ova, approximately equal in number to the brood born previously, has developed and is ready for fertilization. It may be implied from the statement of Kuntz that he assumes *Gambusia* has an annual reproductive cycle in which all the ova for the annual cycle are elaborated at the same time. These interpretations concerning an annual cycle fail to take into account that the pœciliïds arose under tropical conditions and that they have short, often-repeated cycles each similar to one complete annual cycle in a fish of the temperate zone. Under the alternating conditions of the temperate zone these short cycles are repeated as often as environmental conditions permit within one favorable season and then become retarded or cease until favorable conditions are re-established.

The ovarian cycle in *Mollienisia latipinna* and *Mollienisia sphenops* resembles that of *Gambusia*. During the spring and summer broods are born approximately thirty days apart. Ova destined for a new brood reach a maximal diameter of about .7 mm. by the time the embryos of the earlier brood are ready to be voided—about twice the size of the cells in *Gambusia* at the same stage. However, they grow rapidly and reach a diameter of over 2 mm. by the time they are ready for fertilization. Apparently fertilization does not extend over so long a period in *Mollienisia* as in *Gambusia* and the embryos, just after fertilization, do not show the wide range in stage of development characteristic of *Gambusia*.

Pseudoxiphophorus bimaculatus has an ovarian cycle very similar to that of *Mollienisia latipinna* and *Mollienisia sphenops*. However, the interval between broods is longer, being thirty-five to forty days during May, June and July. In an ovary containing a brood ready for birth the next younger group of cells has reached a diameter of only .7 or .9 mm. in diameter. After the brood has been evacuated, however, the cells grow rapidly to a large size, about 2.4 mm., before fertilization.

Lebistes Type.—The rhythm of brood production in this type is very similar to that in the *Gambusia* type. During late spring and summer broods are produced approximately every thirty days. A record of brood production by ten females of *Lebistes reticulatus* is shown in Table I. The data are taken from unpublished records of Dildine and the writer. It will be noted that the most regular production of broods and the shortest interval between broods occur in the late spring and early summer months. In late fall and winter months the interval between broods is nearly as long as in the late spring when broods are produced from twenty-two to thirty days apart. The specimens were kept under fairly constant conditions as regards food, temperature and salt and gas content of the water. No artificial lighting was employed, however, and the exposure of the specimens to light was therefore controlled by the seasonal waxing and waning of daylight. The increase and decrease in brood production paralleled the increase and decrease in daylight. To what extent increased exposure to light may serve as a stimulating agent to reproduction, possibly through the medium of a gonad-stimulating hormone from the pituitary, remains to be worked out experimentally.

Table I

Showing dates of broods produced by ten specimens of *Lebistes reticulatus*. Numbers under months indicate day on which brood is produced. Intervals between broods are expressed in days.

Specimen Number	January	Interval	February	Interval	March	Interval	April	Interval	May	Interval	June	Interval	July	Interval	August	Interval	September	Interval	October	Interval	November	Interval	December	Interval
1											27	25	22	27	18	35	22			46	6			
2											30	22	22	27	18	35	22			44	6			
3							5	29	4	31	4													
4							30	26	26															
5							21	39	29	22	20	26	16	31	16									
6							1	33	4	27	1	34	4											
7									22	28	19	23	13											
8	18																						9	40
9		60	28			39	8	28	6	27	2												20	
10					30			32	2															

In *Xiphophorus* and *Platypœcilus* the brood-bearing record is very similar to that in *Lebistes* with a tendency to a slightly longer period between broods. The same increase in the interval between broods during the winter months has also been noted.

Conditions in the ovary during various phases of the reproductive cycle could be determined only by using the period in which a brood is voided as a first stage and then obtaining regular stages a few days apart up to the time for the voiding of the next brood. Such a series was obtained during the height of the reproductive season. The findings in a number of cases are included here to indicate conditions within the ovary. (1) A specimen of *Lebistes* was sacrificed immediately after it had produced a brood of nine young. No young were found in the ovary and it was evident that all of the brood had been born during a three or four-hour period. In the ovary were found six ova approximately 1.4 mm. in diameter, orange-yellow in color and somewhat transparent. Four additional eggs similar in structure and color but varying in diameter from 0.8 to 1.0 mm. apparently belonged to this group. Presumably all would have been fertilized to form the next brood. Another group of eight cells represented a still younger set which would have been fertilized still later. These younger cells could be distinguished from the older group of cells by their smaller size and difference in structure. The younger group contained cells white in color, opaque and less than 0.5 mm. in diameter with more variation in size than in the older group of cells. It should be emphasized that there were definite gaps in size between the older and the younger group of cells and also between this younger group and a still younger mass of cells in various stages of development. (2) A second specimen was sacrificed and the ovary examined eight days after a brood had been born. Fertilization had recently taken place in a group of ova about equal in number to the brood born eight days before. Some of the fertilized eggs had reached an early blastula stage, some were in early segmentation stages and two had apparently not yet been fertilized. The eggs in the group as a whole varied in diameter from 1.2 to 2.0 mm. One of the larger eggs had not yet been fertilized while several of the smaller ones of the group had been fertilized. Therefore, size, except within the limits indicated, is not an index of the fertilizability of an egg. A second group of cells, about equal in number to the group undergoing fertilization, was opaque and slightly yellow in color. These cells varied in diameter from 0.4 to 0.7 mm. in diameter. (3) The ovary of a specimen killed twenty-one days after the birth of its brood of six contained a new brood of seven, the individuals of which were 5 mm. long. Seven transparent yellow eggs, varying in diameter from .6 to .9 mm., represented the next oldest group. Two were slightly opaque, indicating that they were slightly younger than the others. Fifteen cells, the largest of which was about .25 mm. and the smallest less than .1 mm. in diameter, were white and opaque or had transparent centers and opaque white layers at the periphery.

The development of cells in the ovary up to the time that they become mature and ready for fertilization may be summarized as follows:

1. A large number of transparent cells less than 0.1 mm. in diameter are ready to be drawn upon for differentiation.

2. During the first month some of these cells begin to differentiate and develop a white opaque layer at the periphery. A number of the cells reach a diameter of about 0.5 mm. and become white and opaque throughout. At the same time an older group of eggs and also a developing brood is present in the ovary.

3. During the second month the oldest members of this group of developing cells grow rapidly, become orange-yellow and translucent and reach a diameter of from 0.8 to 1.4 mm. by the end of the month. The number of cells undergoing this development is approximately equal to the number of individuals in a brood.

4. During the third month these orange-yellow translucent eggs comprise the oldest group in the ovary, the embryos of the previous brood having been born. During the first ten days of the month they grow to a maximal size of 2 mm., some being as small as 1.4 mm. in diameter, and at the end of this ten-day period all are fertilized. Variations in the stages of the young embryos indicate that fertilization does not take place in all simultaneously. Fertilization of all members of the group is accomplished within two days. During the remaining twenty days of the month the embryos develop and at the conclusion of the month they are voided. In the meantime younger waves of cells are passing through stages of development already described.

A comparison between the *Lebistes* and the *Gambusia* types indicates: (1) that, at the time of the birth of a brood of embryos, the ovocytes of the next younger group is much larger and better differentiated in *Lebistes* than in *Gambusia;* (2) that the period of time elapsing between the birth of a group of embryos and the fertilization of the next group of ova is shorter in the *Lebistes* type than in the *Gambusia* type.

Data obtained in *Xiphophorus* and *Platypœcilus* were strikingly similar to those obtained in *Lebistes.* The adults of *Platypœcilus* and *Xiphophorus* are larger than those of *Lebistes* but ova of the same age are equal in size. However, in *Lebistes* fewer eggs come to maturity at one time and the broods contain fewer individuals. Other species which have cycles like that of *Lebistes* or intermediate between the *Lebistes* and the *Gambusia* type are *Pœcilia vivipara, Micropœcilia picta, Micropœcilia paræ, Brachyrhaphis episcopi* and *Limia vittata.* Others not yet studied will undoubtedly be added to the list.

Quintana atrizona Type.—In this type there is a very short interval between the birth of a brood and the fertilization of the ova of the following brood. In a female killed and examined twenty hours after the birth of a brood, the next group of cells in the ovary was found to be fully developed. These ova, approximately equal in number to the brood just born, were 1.8 to 2.2 mm. in diameter. Some had apparently been fertilized a few hours before. Of this group—nineteen in all—seven had reached an advanced segmentation stage, nine were in earlier segmentation stages and three had not yet been fertilized. It was apparent that fertilization had taken place in most of the ova immediately after the evacuation of the previous brood and that fertilization in the remaining three cells would have been complete within a few hours more. Differentiation and growth of the ova next in line for fertilization had reached a final stage by the time the embryos of the previous brood were born and the interval between the two events was reduced almost to the vanishing point.

TABLE II

Illustrating production of broods in *Pœcilistes pleurospilus*

Date	No. of young in brood	Interval in days between broods
November 20	2	
December 16	2	26
January 8	2	23
February 1	2	23
February 21	3	20
March 15	3	22
April 3	7	19
April 25	7	22
May 10	8	16
May 24	9	14
June 5	9	12

If the ova should develop still more rapidly and reach their final stage of differentiation and growth before the older brood of embryos was evacuated a condition would exist in which it would be possible for fertilization to occur before the older brood had been evacuated and for two broods to exist in the ovary at the same time. Such a condition of superfetation does occur in the next species of pœciliids to be considered.

Reproductive Cycles Involving Superfœtation

Pœcilistes pleurospilus and Pœciliopsis infans.—Specimens of *Pœcilistes pleurospilus* were observed from November to May in order to secure records of the broods produced. Single specimens were then sacrificed at different intervals after the production of a brood to determine the state of development of embryos and ova within the ovary. The record of broods produced is shown in Table II. The record shows that: (1) the interval between broods was longest in the winter months and that it was becoming shorter in the early spring months; (2) the broods were becoming larger as the spring advanced; (3) in general, also, the broods were smaller than those of pœciliid fishes in which superfetation does not occur; (4) during the spring months the interval between broods is much shorter than in *Lebistes.*

Stoye (1935) finds the interval between broods in this species to be ten to twelve days. This is apparently the record of specimens reproducing in May or June.

A female of *Pœcilistes pleurospilus,* killed two days after the last brood of eight young had been born, contained two broods of unborn embryos in the ovary. The older brood consisted of nine embryos which were well developed but far from the stage in which they would be born. The eyes were well developed and pigmented, and pigment cells were present over the brain, down the mid-dorsal line and along the lateral line. All embryos were in approximately the same stage of development. The second brood contained nine very young embryos, with only a few somites developed. A group of twelve cells about .5 mm. in diameter, together with many smaller undifferentiated cells, were also present in the ovary. Another female, sacrificed twelve days after she had produced a brood, contained an ovary in which two broods were well separated in age and also a group of developing ova nearly 2 mm. in diameter.

An abundance of preserved material of *Pœciliopsis infans* was available and forty-two ovaries were dissected. In every ovary two broods of embryos were found, together with a group of cells approximately equal in number to the embryos in a brood, well in advance of the development of a mass of smaller undifferentiated cells. Embryos of a single brood varied somewhat more in their degree of development than was the case in *Pœcilistes pleurospilus* but the least advanced member of the older brood was always separated by a considerable gap from the most advanced member of the younger brood.

The condition found by Henn (1916) in *Pseudopœcilia fria* and *Diphyacantha chacoënsis* indicates that the ovarian cycle of these forms is probably like that of *Pœciliopsis infans* and *Pœcilistes pleurospilus.* Henn dissected five ovaries and found in each a brood of large embryos, a second brood of small embryos with about the same number of individuals as in the first group and a small number of developing ova.

In the first two of these species, at least, the sequence of events for development of ova and embryos would be as follows: (1) a group of ova grow and differentiate until they reach a diameter of about 2 mm. At this point an older brood of embryos, *A,* is at the point of being born and a much younger brood, *B*, is also present in the ovary; (2) the ova are fertilized, forming brood *C*, and the older brood, *A*, is born at practically the same time; (3) broods *B* and *C* develop for a period of 10 to 22 days and during this time another group of younger ova develop to the maximal size; (4) brood *B* is born, brood *C* is retained in the ovary and the eggs of the new group are fertilized to form brood *D*.

Superfetation, as observed in the species mentioned above, is apparently caused by the earlier development of the younger ova, allowing earlier fertilization. A sequence might be arranged from the species described so far, illustrating regular stages through which superfetation might arise. (1) In *Gambusia* and *Mollienisia* the ova remain quite small until the birth of the brood already contained in the ovary; they may not reach a state in which they can be fertilized for about two weeks. (2) In *Lebistes* the developing ova are much larger and better developed when the brood in the ovary is born and this continued growth brings them to the point of fertilization a few days earlier. (3) In *Quintana atrizona* the ova have reached a still more advanced stage at the birth of the brood in the ovary; they have reached their maximal size and complete differentiation and fertilization takes place immediately. (4) The ova in *Pœciliopsis infans* and *Pœcilistes pleurospilus* attain their full size and complete differentiation long before the brood in the ovary is born. Fertilization of the ova takes place and the resulting embryos are retained in the ovary as a second brood.

Heterandria formosa.—This species has small broods with short intervals between the broods.

The records shown in Table III are those of two young females that have just attained sexual maturity. The most important features in the records are: (1) the broods are very small, one or two embryos being produced at a time during the winter months and as many as five or six at one time during May and June; (2) the interval between broods is long during the winter months, thirty-three to forty-one days. It becomes shorter with the approach of spring, reaching the shortest interval —three to eight days—in May and June. Seal (1911) made a record of broods produced by larger specimens during the months of July and August. He found that the broods containing from four to sixteen embryos were born at intervals of from four to nine days.

A female which had produced a brood of nine was sacrificed and examined on May thirteenth. The ovary was well filled with embryos and when these were dissected out of their follicles and arranged in groups it was found that six levels of development could be distinguished. There were nine embryos at the oldest stage (brood *A*) and these would presumably have been born about seven days later. All external parts of the embryos were well developed except the caudal fin, and the yolk sac was almost completely absorbed. They were about 5 mm. in length (snout to base of caudal fin). Eight embryos formed

TABLE III

Brood production in *Heterandria formosa*

Specimen No. 1

Date	No. of young in brood	Interval in days between broods
December 12	1	
January 24	2	33
February 9	2	16
March 1	2	19
March 17	2	16
March 29	3	12
April 6	2	8
April 14	3	8
April 26	2	12
April 30	3	5
May 4	2	4
May 12	5	8
May 20	3	8
May 26	2	6
June 2	3	7
June 8	2	6
June 14	3	6
June 17	5	3
June 20	4	3
June 28	3	8
July 1	1	5
July 11	2	10
July 25	1	14

Specimen No. 2

Date	No. of young in brood	Interval in days between broods
December 10	2	
January 21	2	41
February 7	2	17
February 28	2	21
March 6	1	6
March 17	3	11
April 4	2	18
April 7	2	3
April 20	2	13
April 26	3	6
April 30	1	4
May 9	3	9
May 12	2	3
May 20	2	8
May 26	6	6
May 31	4	5

brood *B*. The members of this brood were considerably younger than those forming brood *A*. There were eight embryos in brood *C*, nine in brood *D*, eight in brood *E* and at least six in brood *F*. It is possible that a still younger brood was present. The largest unfertilized cells were about .7 mm. in diameter and it is evident that fertilization would occur when the cells had reached this size.

The only peculiarity in the history of the ova up to the time of fertilization lies in the fact that they are very much smaller at fertilization than the ova of the other species described. This fact would indicate that the larger quantity of yolk elaborated in the ova of the others is not required in *Heterandria formosa.* If the larger amount of yolk is not formed and yet the embryos go on to the more advanced stage while being retained in the ovary, it must be assumed that the follicle cells surrounding the developing embryos are responsible for furnishing the food materials. The expanded yolk sac, containing little yolk, is in a position to absorb the food materials, for it completely surrounds the embryos in the earlier stages, becomes very vascular, and is in contact with the walls of the follicle almost up to the time of birth.

Both viviparity and superfetation in this species have been carried to an extreme degree and it is quite likely that there will be found in some small pœciliïd fish a stage intermediate between that of *Heterandria formosa* and that represented by *Pœciliopsis infans* and *Pœcilistes pleurospilus.*

It is reported by Stoye (1935) that the mode of reproduction in *Priapella bonita* and in *Phalloptychus januarius* is similar to that in *Heterandria formosa*, one or two young being born at a time at intervals of a few days.

It is also reported by Stoye that in *Micropœcilia branneri* the young are born one or two at a time every few days. This observation would tentatively class this species with *Heterandria formosa* in its mode of reproduction. A most interesting situation is presented in this case since it has been stated by Stoye and verified by the writer that two other species in this genus (*M. paræ* and *M. picta*) produce broods of thirty to forty embryos at intervals essentially like those of the *Lebistes* type. Unfortunately no breeding specimens of *Micropœcilia branneri* have been available and gravid ovaries have not been examined.

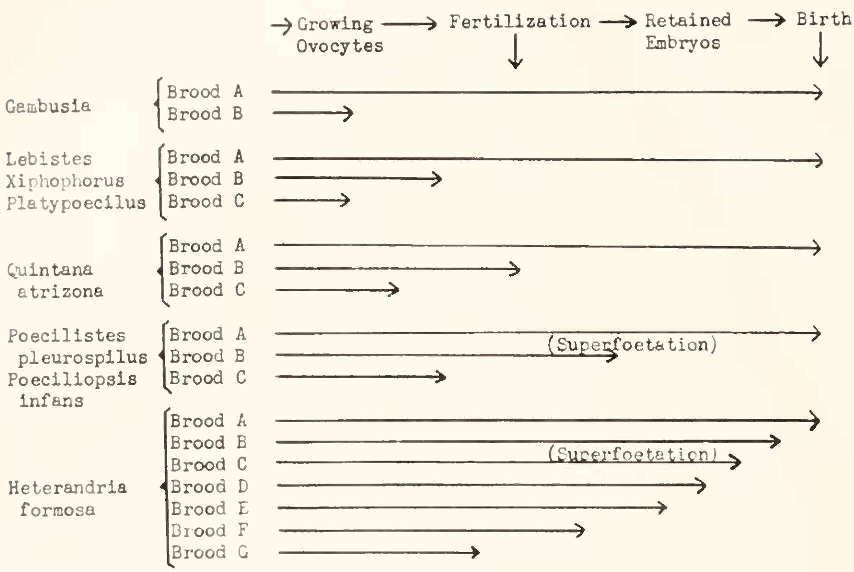
Summary

A graphic summary of the relations of growing ovocytes, mature ova, the fertilization process, the broods of embryos retained in the ovary and the time of birth is offered in Table IV. Each group represents the situation within a single ovary at the height of the reproductive season and at the moment in which a brood is born. The term brood is used to indicate a group of growing and differentiating ovocytes of approximately equal development up to the time of fertilization and also the

embryos produced by the fertilization of these ova up to the time for birth. No quantitative relations are implied concerning comparative size of ovocytes or embryos in different species nor of comparative lengths of time that embryos are retained in the ovary.

A study of Table IV leaves one with the impression that the extreme type of superfetation in *Heterandria formosa* might have arisen from the simpler type as illustrated in *Pœcilistes pleurospilus*. The argument also appears to be defensible that the simpler type of superfetation found in *Pœcilistes* might have arisen from a condition like that illustrated by *Quintana atrizona* in which a new group of ova are ready for

Table IV



fertilization when a brood is born but in which fertilization is delayed for some hours. All of this implies that the extreme cases of superfetation should be considered as the specialized and derived types while those without superfetation are the simpler. Since the Pœciliidæ undoubtedly arose from an oviparous cyprinodont such an assumption does not seem to be overdrawn. That group of the Pœciliidæ (*Gambusia* and *Mollienisia*) with the ovarian cell cycle which most resembles the oviparous cycle may by the same sign be considered the most unspecialized.

The evidence which now exists concerning the evolutionary relations of the different genera of the Pœciliidæ gives no support to the thought that those genera in which superfetation occurs are more closely related to each other than to any other genera. Rather, it appears that superfetation has arisen more than once and that the present cases represent convergent evolution. The occurrence of superfetation therefore has no taxonomic significance.

The Control of Reproductive Cycles

The factors which control the rhythms of reproduction in this group of fishes are presumed to be the same as for other fishes and are probably also the same group of factors which control the cyclical production of ova in higher vertebrates. These factors would be in part environmental, in part the driving influence of the gonad-stimulating hormones of the pituitary and, finally, an hereditary factor which would place a limit within which the others might act.

Annual Rhythm

In most of the laboratory-reared pœciliïds in which an annual record has been kept, it has been noted that the number of embryos in a brood and also the number of broods produced increases in early spring, reaches a climax in late spring and early summer and declines in late summer. Production of young falls to a low level or ceases altogether during the winter. Studies on *Gambusia affinis* by Barney and Anson (1921*a*), Hildebrand (1917) and Kuntz (1913) show that the same type of rhythm is present in specimens studied in the field under natural conditions. In the laboratory specimens reared by the writer, food and temperature have been maintained at a constant level. In the natural habitat, both temperature and food supply have some effect on the rhythm. The laboratory-reared specimens have been subjected to one variable factor, namely light. No attempt has been made to regulate the length of time each day during which specimens were exposed to light and consequently they have been exposed to the usual seasonal fluctuation. The highest point in the reproductive cycle coincides with the longest daily exposure to light and the lowest point in the reproductive rhythm to the shortest daily exposure. This coincidence would seem to be significant in view of the fact that seasonal variation in light has accompanied seasonal variation in the reproductive cycle in both wild and laboratory specimens. It might be tentatively postulated that light acts directly to stimulate the reproductive organs differentially but more likely through the gonadotropic hormones of the anterior pituitary.

Brood Production

Apparently the factors which control the size of broods and the rate of brood production are in part the same as those concerned with the high and low phases of the annual cycle. The contrast between the reproductive activity exhibited by an ovary of *Heterandria formosa* at the height of the reproductive season and one at a low ebb is striking and demonstrates clearly one of the factors responsible for brood production. In the ovary at the height of the breeding season there are five or six broods of several individuals each at different levels of development. At a still lower level are one or two groups of cells growing to the size necessary for fertilization. Every few days a brood is born and all levels of embryos and younger fertilized cells move up to a higher level. The entire picture is one of the crowding up of the maximal number of cells from the lower levels with a stimulating factor driving them, with the result that larger broods are produced and the intervals between them decreased. The reverse of this picture is seen in the ovary of specimens of this same species during the winter. Small numbers of ovocytes are developing and if broods are present in the ovary there are rarely more than two and they consist of one or two embryos each with a wide gap between them in level of development. The impression is created that the cause for the infrequency of broods, the small numbers of embryos in broods, and the paucity of developing ovocytes, lies in the lack of a stimulating agent. As suggested before this agent is probably a follicle-stimulating hormone from the anterior pituitary.

An influence retarding the development of the younger ovocytes is also present. This is best seen in the ovarian activity in species without superfetation. In *Gambusia*, for example, the younger ovocytes will develop to a diameter of only .3 mm. until the brood of embryos held in the ovary is released. Then within a few days they more than triple their diameter. In *Lebistes* and *Xiphophorus* the retarding effect is not so marked and ovocytes become much larger (0.8 mm. to 1.0 mm.) before a brood is discharged. In *Quintana atrizona* retardation by the growth of embryos is still less and the ova reach the maximal size and are ready for fertilization before the previous brood is evacuated. However, the retention of a brood of embryos seems to act as a block upon fertilization; otherwise some cases might be found in which the fully matured ova had been fertilized before the discharge of the older brood of embryos. Although the block upon fertilization of a new group of ova exercised by the presence of a brood of embryos seems to be removed in *Poecilistes pleurospilus*, it is apparently partly operative for not more than two levels of broods are found in the ovary at one time.

The presence of older ova also serves to retard the development of younger cells. In an old virgin female a group of ova will have reached the maximal size and younger groups of cells approximately equal in numbers will be found at lower levels. If fertilization is prevented, the younger groups of cells will not develop and the condition may prevail for months. When the older group of ova is fertilized and the embryos begin to develop, the block is released and when the embryos are discharged the younger waves of cells will advance.

From the above observations the controlling mechanism might be postulated as follows: (1) a hormone stimulates the ovary to produce waves of developing ovocytes. The production of the hormone or its effectiveness is acted upon by light and possibly other factors in the environment. (2) A retarding influence upon the growth of younger cells is exercised by the presence of older cells and by broods of retained embryos. (3) A balance has been struck between the two influences and is fairly constant within a species so that the rate of brood production at any season is practically constant. Hereditary factors control this balance. (4) The balance between the stimulating and retarding influences varies with the season, the stimulating agent being most effective in spring and early summer and least operative during the fall and winter months.

Relation of Broods to Annual Cycles in Other Fresh Water Fishes

Some of the fresh water fishes of the temperate zones have annual life cycles, maturing their gametes within the year and dying before another season of reproduction. This type of life cycle, described by Hubbs (1921) in *Labidesthes sicculus,* is not common. Other fishes bring their ova to maturity at the end of their second or third year or later and a new generation of ova to maturity each year thereafter as long as they live. In some fishes the ova have been two years in coming from ovogonia to the full size and complete differentiation necessary for fertilization. *Cottus bairdii,* described by Hann (1927), represents this type. It has been observed by the writer that *Perca flavescens* in Lake Michigan follows the same course in developing its ova although there is evidence that *Perca* requires sometimes three years before the first batch of ova is matured. In these fishes of the temperate zone a marked retarding influence is exerted by the cold weather of late fall and winter. Under more favorable food and temperature conditions such as can be maintained in the laboratory, an earlier maturing of gametes can be secured indicating that the reproductive rhythm occurring in nature is not the maximal one. These same fishes under tropical conditions would presumably produce more young in a shorter space of time although inherent factors would limit the rapidity of the occurrence of cycles.

As already indicated, the Pœciliidæ arose in a tropical climate (Eigenmann, 1906) and some genera have migrated into the north and south temperate zones. Their pattern in reproductive cycles has apparently been fixed in the tropics under most favorable conditions for the production of the maximal number of cycles in the shortest space of time. Apparently other inherent factors associated with viviparity have had some influence in shortening reproductive cycles as oviparous fishes like the cichlids, which have been associated for a long time with the Pœciliidæ in geographic distribution and climatic conditions, do not have such short cycles.

The production of successive waves of ovocytes in the Pœciliidæ is interpreted as parallel to the seasonal waves produced in such forms as *Cottus* and *Perca*. It takes two years to produce a batch of mature ovocytes in *Perca* and *Cottus* and only a few months in the pœciliids. If any pœciliid should produce a single batch of ova, bring them to maturity and fertilization and void the embryos without bringing any more ovocytes to maturity, we should have a case parallel to that of *Labidesthes*, but apparently none exists. The interpretation of each pœciliid short cycle as equivalent to the long cycle of *Cottus* and *Perca* instead of a long-drawn-out annual cycle, as suggested by Barney and Anson and Kuntz, rests upon the question of whether there is synapsis in residual gonial cells on a large scale to produce the entire season's potential ova or synapsis of only enough residual gonia to form the ova for a single brood. If synapsis occurred on the necessary large scale and only once a year, followed by the maturing of small groups of ovocytes, an annual cycle such as occurs in *Cottus* and *Perca* might be postulated. However, during the height of the breeding season small waves of synapsis of gonia occur at regular intervals and new ovocytes are being added at the lower levels as the ones at the upper levels reach maturity and are fertilized.

Summary

1. All species of the Pœciliidæ are ovo-viviparous.

2. At the height of the breeding season broods are produced at regular intervals varying from about forty-five days in some species to five or six days in others.

3. In laboratory-reared specimens and in specimens breeding in temperate zones the shortest interval between broods occurs in spring and early summer and the longest interval during fall and winter.

4. In the reproductive cycle of *Mollienisia* and some other species, the oldest group of ovocytes remaining in the ovary just after a brood has been extruded is very small. Growth is rapid and the ova are ready for fertilization about eight days after the voiding of the last brood of embryos.

5. In *Lebistes* and other species with similar reproductive cycles, the oldest group of ovocytes remaining in the ovary just after a brood has been voided are much larger than in *Gambusia* and fertilization occurs a few days earlier than in *Gambusia*.

6. In *Quintana atrizona* the oldest group of ova remaining in the ovary are fully developed and ready for fertilization at the time of birth of the previous brood of embryos.

7. Superfetation occurs in *Pœcilistes pleurospilus* and several other species. Fertilization of the oldest group of ova takes place before the voiding of the brood already in the ovary and two broods at different levels of development are found in the ovary.

8. In *Heterandria formosa* and possibly other species there is an extreme development of superfetation with six or more small broods at different levels of development occurring in the ovary at one time at the height of the breeding season.

9. Older groups of ova and embryos retained in the ovary retard the development of younger groups of cells and a balance is maintained between this retarding influence and the agent, assumed to be a follicle-stimulating hormone from the pituitary, which forces the cells to grow from the lower to the upper levels of development.

10. The short reproductive cycles of the Pœciliïdæ are the equivalent of the longer annual or biennial reproductive cycles of the fishes of the temperate zones.

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